Summary of items identified for correction or possible revision during EPA's initial evaluation of the chloroprene PBPK model, the in vitro chloroprene metabolism modeling, and associated documents and files

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March 20, 2020

The U.S. EPA has reviewed the modeling of the in vitro metabolic data, in-vitro to in-vivo extrapolation (IVIVE), and PBPK modeling of chloroprene presented in the report from Ramboll and supplemental materials provided in July 2019 and corresponding computational model files (model package), consistent with EPA's standard quality assurance (QA) process for PBPK models. Although EPA has provided feedback on that version of the model package during this review, this is not a formal QA review.

Following is a collection of all items of concern or requiring correction that were identified and communicated by email with Ramboll and Denka, primarily between September 10 and December 20, 2019, plus new discussion of the in vitro sample volume calculation on page 5, reflecting a recent realization.

In some cases, this review expands a bit on issues related previously by email to clarify the concern. For the most part plots that accompanied the original emails have not been copied here, but they can be provided upon request.

Email correspondence from Ramboll indicated that a number of these have already been addressed, but they are included here for completeness of record. There are also some items for which Ramboll's reply indicated that they would not make changes but are likewise included here to be comprehensive in listing the issues identified during the QA.

Finally, a few items are presented as suggestions in the event that future work is conducted. For example, the accuracy of VVIAL is not supported by the data and suggestions are included here as guidance for additional uncertainty analysis that EPA may conduct.

PBPK chloroprene.model file, QRC: equation is correct to calculate by subtraction, but not constrained to be > 0. Could result in an error if volumes of other tissues are set too large.

PBPK chloroprene.model file, KFLU and KFKI: in the IVIVE Excel spreadsheet, the in vitro kf (L/h/mg MP) is multiplied by MPPGLU (mg MP/g lung), then by the lung fractional weight (no units) and a conversion factor of 1000 g/kg, to yield a value with units of L/h/kg BW. In the PBPK code section before the Dynamics block, the units are listed as "L/h/BW^0.75", which is incorrect, but the calculation multiplies the constants by $BW^{1.0}$. The units listed in the model code should be consistent with the IVIVE extrapolation; if the extrapolation is not changed per the following point, then just put "L/h/BW".

Note: this is a departure from the usual assumption that clearance scales as $BW^{-0.25}$ and seems inconsistent when Vmax values are scaled by $BW^{0.75}$. EPA believes that scaling as $BW^{-0.25}$ is more consistent with general practice in PBPK modeling, but the difference in this application may be small. In particular, if the IVIVE calculation spreadsheet and PBPK model just use 70 kg for a human adult, the scaling won't matter for predictions for the average adult, but it may matter for predicted animal doses

if the animal BW in the study differs significantly from the assumed standard BW and also if model estimates are ever extended to children or adults with body masses substantially different from 70 kg.

Figure 4 in draft report could not be reproduced, appears not to have been updated with final parameters. Also, the model results being plotted were for the venous blood leaving the lung compartment (CVLUM), which is 20% higher than the mixed venous blood (CV). In the plot below the circles are CVLUM, the line CV. Blood samples are usually assumed to be CV.

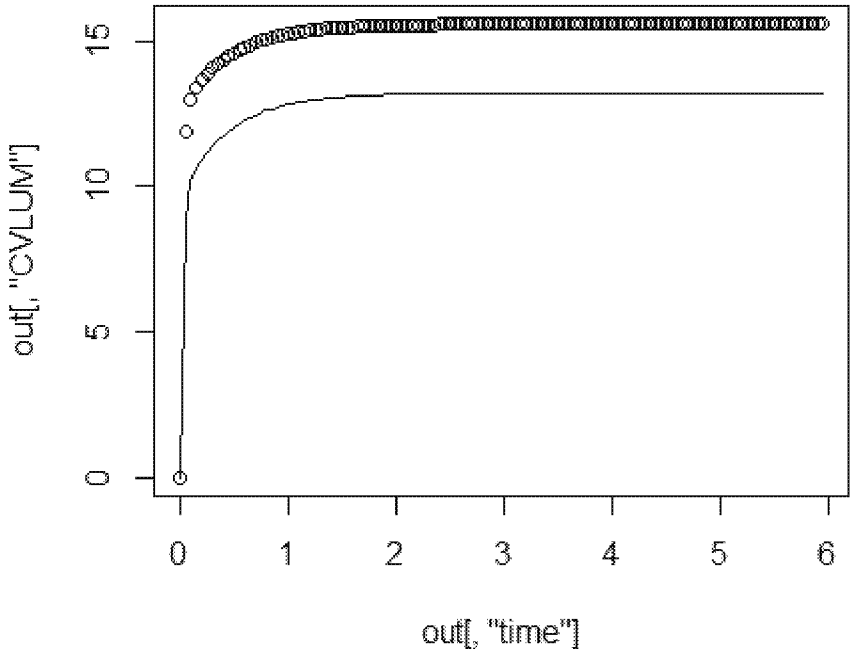


Table 1 and small table on p. 21: clarify /correct that the lowest concentration in the in vivo PK time-course study is 12.3 ppm. The lowest concentration in the bioassay was 12.8 ppm, so that's the correct value for internal dose calculations.

OBSERVATION REGARDING QPC/QCC: In the model report section on plethysmography, the average ventilation measured is 56.2 mL/min in 22 g mice. The QPC is then calculated using an assumed ratio of 2/3 for alveolar/total ventilation: QPC = 39.4 L/h/kg$^{0.75}$ given that value. Then QCC = 27.2 L/h/ kg$^{0.75}$, using QPC/QCC = 1.45.

RECOMMENDED CHANGE: Ensure that values in Fmouse_InVivo.R and FMouse_metric.R are set to use corresponding study-specific values or values based on an appropriate standard QPC for mice.

BW: Table S-1 lists BWs for mice and rats as 0.03 and 0.25 kg, citing Brown et al., same weight for males and females. But the IVIVE calculations (Supp D, "IVIVE" tab) use 0.035 and 0.04 kg for female and male mice, and 0.33 and 0.45 kg for female and male rats, respectively. The discrepancy vs. default value range from 17% to 50%. Ideally these should be the BWs for the animals from which the tissues were

collected for the in vitro studies, and this should be stated in the spreadsheet comments. Female mice are most important; BWs may be in laboratory report. Otherwise BWs in IVIVE calculation should match values in Table S-1.

The _metric scripts use the default BW values in Table S-1. So, BW^0.75 scaling is then being used to scale metabolism to the standard BW used to calculate the internal metrics, or 22 g of the animals used in the in vivo dosimetry study.

Study-specific BW values for the tox studies being analyzed should be used for _metric calculations rather than default BWs. Preferably the report table which lists the metrics calculated would also list the animal BW values used, but it is sufficient to provide comments in the model script.

Liver and lung microsome content, Supplement D, IVIVE tab, cells G22-G27 (liver) and cells H22-H26 (lung in all species):

o Mouse liver: From Supp Mat C, value of 35 mg/g is from Medinsky et al. (1994); cell G27 says "rat value used for mouse", but rat value is 40 mg/g.

o Rat liver:

Main report p. 9 says 45 mg/g used for rats, not consistent with 40 in IVIVE spreadsheet (cells G24-25);

Supp Mat C says an average of values for rat from Medinsky et al. (1994) (sentence is confusing, "For mouse, 35 mg/g liver was reported by Medinsky et al. (1994) for both rat and mouse,") and 45 mg/g from Houston and Galetin, but it's not entirely clear why a cross-species average would be used for the rat, but not the mouse; if Medinsky et al. (1994) also measured 35 mg/g from rat liver, then an average may make sense...

- In Barter et al. (2007), Figure 2, part A, there appear to be many papers reporting 45 mg/g for the rat, so the value of 45 mg/g appears to be better supported;
- If so, then IVIVE spreadsheet should be changed from 40 to 45 mg/g
- reference in cell 27 just cites Houston and Galetin (2008) for rat, not consistent with "40".

o Human liver:

- Text in main report, p. 9, says 40 mg/g, which matches the value listed in Supp Mat C
- IVIVE cell G26 has 50 mg/g
- Supp Mat C, "Based on their meta-analysis and consensus report of the human data (Barter et al., 2007), 40 mg/g liver is recommended for human adults for chloroprene IVIVE-PBPK modeling," so it would be less confusing if the main report and IVIVE cell G27 cited this reference, not Barter et al. (2008)
- From Barter et al. (2007): "Values of MPPGL were approximately 36 and 31% lower in newborn and elderly (80 years) individuals than those in a 25-year-old individual (typically the age of individuals used in clinical pharmacology studies). The use of a value of MPPGL of 40 mg g-1, determined for a young adult, would be expected to result in an overprediction of clearance in very young or very old patients. Therefore, MPPGL values relevant to the age of the population in which predictions are being made should be used in IVIVE."
- The young-adult value of 40 mg/g likely will be most health-protective for this application.

- But the statement in Supp Mat C appears to mis-represent the conclusions of Barter et al. (2007): it should be made clear that this value is the recommendation of the model authors, not the cited paper.

o Human lung: value of 23 mg/g in cells H22-26 matches Himmelstein et al. (2004b), but text in the report says 20 mg/g, and this is the conclusion after some discussion in Supp Mat C. Use of 23 mg/g is more health-protective, but discrepancy between the report text and value/footnote in Supp Matt D (IVIVE tab) should be resolved.

IVIVE In Vitro Values of KFLUC for female rat (cell V33) and male rat (cell V38): These cells have calculations which are not explained and do not take values from the in vitro metabolic results; e.g., "=1.2/(0.82*2)/1000" in cell V33, which should be just equal to Parameter_Summary cell I18.

VVIAL value used in various scripts: From IISR-17520-1388, p. 11, "The total volume of Gerstel 10-mL vials used for the incubations was confirmed by gravimetric displacement with water. The measurement was made on 2 occasions once for the liver and lung microsome incubations (n=10 vials) and once for the kidney microsome incubations (n=10 vials). The respective mean (±SD) weights when filled completely with water at room temperature were 11.648 (±0.222) and 11.634 (±0.051) grams." Based on this description, the vial volumes are only accurate to ~ +/- 0.1 mL; the values used in the script are specified to a higher degree of accuracy than warranted. VVIAL for the Himmelstein et al. (2004) study is also likely only accurate to +/- 0.1 mL. While the difference is unlikely to have a significant effect on most of the parameters estimated, any re-estimation should use 11.6 mL for experiments described by Yang et al. (2012) and 12.0 mL for experiments described by Himmelstein et al. (2004).

Results spreadsheets for MCMC analysis: Female Rat Kidney plot leaves out the lowest concentration data and simulation results, initial value = 0.09 uM. The results underpredict metabolism a bit at that lowest concentration, though the fit to the whole data set is good. The figure should be updated to include results at the lowest concentration.

Variance in background loss rate: A linear-y plot of the same results showed that for the highest concentration data/simulation, the amount attributed to system loss, ARLOSS, is 5.9 times the amount attributed to metabolism. A similar result occurs for the female mouse lung highest concentration. The fact that the concentration decline predicted by the model is faster than shown by the data suggests that the actual loss rate was lower in the experiment. (It is not likely that this result is due to a lower rate of metabolism.) This suggests that in any future re-estimation of parameters, it would be better to capture the full variance in the loss rate based on the distribution among the control incubations. Also, specifying RLOSS to 4 significant figures implies a level of confidence not reflected by the control data, which showed that the total loss in a single incubation (including sampling losses) after 1 hour varied roughly between 10 and 20%; i.e., a factor of +/- 33% of an average loss of 15%. (1.33 x 15% = 20%; 0.67 x 15% = 10%.)

Background loss rate in Himmelstein et al. (2004) vs. Yang et al. (2012): The loss rate RLOSS was estimated using data from Yang et al. (2012), for which a larger number of control incubations were run, and the raw data are available from the lab report. This value is assumed to apply to the data from Himmelstein et al. (2004). Since human liver metabolism was only measured/reported in the Himmelstein paper and different vials were used, it is suggested that model simulations of control experiments using the estimated loss rate (distribution) be compared to the control data from

Himmelstein (digitized from the available plots) to assure that the loss rate was not significantly different in the Himmelstein experiments.

Units of background loss: Units for RLOSS should be L/h; at one point in the report it was listed as L/hr/g.

Model compartment for background loss term: in the original model, which assumed that air and liquid phases were at equilibrium at all times, the background loss term was coded as a rate proportional the concentration in the liquid phase (CM1 = CA1*P1). When the in vitro model code was revised to explicitly describe the two phases, the rate was written as RLOSS*(CA1*P1). When samples are taken from the vial, the air pressure in the gas phase would be temporarily reduced, so it is more likely that air is leaking into the vial than leaking out, so the assumption that losses occur in the medium seems most likely. The impact is likely not significant, but if further refinements are made it is suggested that the loss rate should be calculated as RLOSS*CM1 and the loss term should occur in the differential equation describing the amount in the liquid phase rather than in the differential equation describing the air phase.

Sampling volume in for various experiments (VINJ): The likely source of the value of 385.8 uL for sample volume used for some but not all model scripts/analyses of the Himmelstein et al. (2004) experiments has been identified.

When a sampling syringe is inserted into a vial and the syringe plunger pulled to take the sample, but before the syringe is withdrawn, the volume of air in the incubation headspace **and** syringe are in that moment connected. If the volume of the headspace is Vair and the volume taken into the syringe is Vsamp, then the volume of air into which the air-phase chloroprene is distributed is increased to Vair + Vsamp, which would slightly dilute the chloroprene concentration by a factor, Vair/(Vair + Vsamp). If the concentration of chloroprene in the air before the syringe is filled is Cair, the concentration in the headspace and syringe after filling would be Cair*Vair/(Vair + Vsamp), and the mass of chloroprene taken into the syringe would be Cair*Vair*Vsamp/(Vair + Vsamp). Using Vair = 10.96 mL (volume for many of the Himmelstein et al. incubations) and Vsamp = 0.4 mL (400 uL), Vair*Vsamp/(Vair + Vsamp) = 0.3859 mL or 385.9 uL.

While 385.9 uL is still slightly discrepant from 385.8 uL, it seems reasonable to assume that the number was introduced to account for the decrease in pressure and increase in volume in the air phase being sampled as a syringe is being filled. The difference between 385.9 uL and a nominal sample volume of 400 uL would be most critical when analyzing low-activity samples such as the human lung, so it could be that the value was only refined to reflect the dilution effect for those model scripts.

However, EPA's recommendation is that calculation shown above be explicitly included in the in vitro model code, to make the correction transparent, and that the sample injection volume used in the calculation be set to 400 or 200 uL (0.0004 or 0.0002 L) as appropriate for each experiment. This will also have some impact on the analysis of the Yang et al. (2012) data, for which the sample volume was 200 uL. The largest relative impact would be on estimated human and rat lung metabolism, and estimated kidney metabolism, because the sampling loss is a larger proportion of the total concentration decline in those experiments.

MCMC Analysis and Results

The model scripts (code) and results in the 'MCMCoutput' folder have not been evaluated for this QA. If, based on the results of the peer review, there is a decision to use the analysis as presented by Ramboll, it will need to be QA'd at that time. EPA is conducting an initial independent statistical analysis of the control incubation data at this time, with plans to extend that analysis prior to model implementation. A comparison of the results from these analyses with Ramboll's will provide a higher-level quality check; e.g., if there is close agreement between the results of EPA's analysis and Ramboll's, that will provide a greater degree of confidence.

Data Review

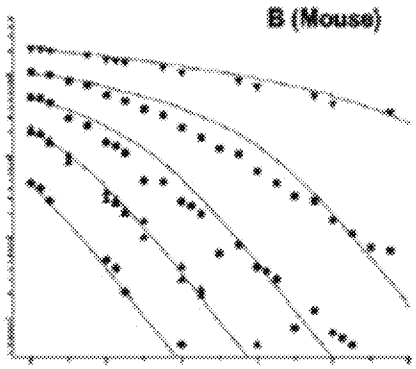
There is an increasing expectation for EPA to provide quantitative uncertainty analysis of the models it uses, including PBPK models. To perform such an analysis for the chloroprene PBPK model, good estimates of uncertainty in the model parameters should be used. For the metabolic parameters, a full evaluation of uncertainty depends on accurate representation (inclusion) of the in vitro PK data. While a good estimate of mean or maximum likelihood parameters may only require that the data set be correct on average, an accurate estimate of parameter uncertainty depends upon also quantifying the variance among the data. In particular, the discrepancies noted below between the data tables used in the model scripts and parameter estimation are not likely to significantly affect the estimated mean values for the corresponding parameters, but we encourage that they be corrected for subsequent uncertainty analysis.

Yang et al. (2012)

The IISR report includes instances of repeat or duplicate incubations. For example, there is a repeat of the 1 ppm incubation for the female mouse lung, but the data used in the analysis and shown in the plots of model fits are average values for the two incubations. From scanning the IISRP report it seems that within each species/gender/tissue set of incubations (e.g., male mouse kidney) there is at most one incubation concentration where the incubation is repeated; for the majority of the data only a single incubation is run for each concentration. Because the model vs. data plots only show means of repeated incubations, the extent of this variability can't be seen in those plots.

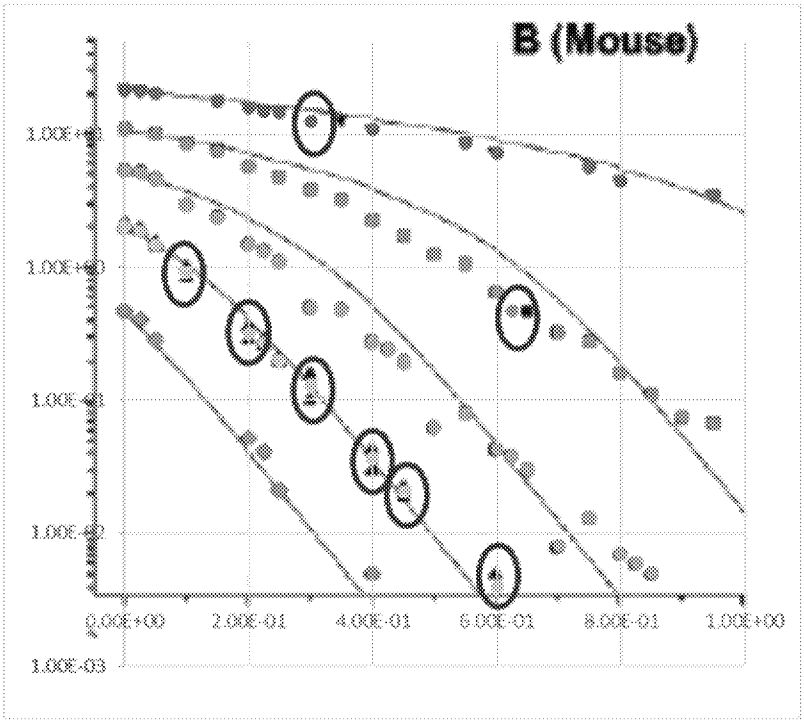
Himmelstein et al. (2004)

Himmelstein et al. (2004) used staggered start times and the data show clear variability between vials. An example is just below; for the middle set of data the variability is particularly pronounced. While the original data tables are not available, it is suggested that the results for each incubation vial should be identified based on the assumption that the sampling interval is consistent at 0.2 h, to allow for analysis of between-vial variance as a component of parameter uncertainty. Since the human lung data show only a single point at each sampling interval, and Himmelstein et al. (2004) otherwise plotted each measurement separately, it is presumed that one incubation vial was used for each initial concentration of the human lung CP oxidation experiments.

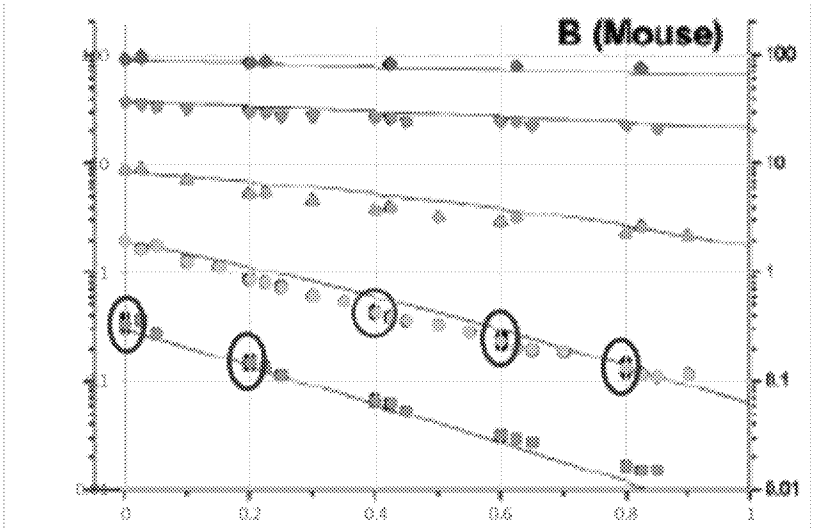


Male mouse liver oxidation data from Himmelstein et al. (2004)

For the male mouse liver and lung, there are discrepancies between the numerical data and the figures that are likely not an artifact of digitizing the data (assuming the data were recovered from the published figures this way). Plots are copied below. There are a couple of points where the sample time is wrong and a number where it appears that an average value is used to replace multiple points at the same time.



Male mouse liver data QA



Male mouse lung data QA